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Relationship between Circulating Immune Complexes, Serum Interferon and Clinical Features in Sarcoidosis

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Abstract. In order to assess the importance of circulating interferons and immune complexes as hypothetical mediators of some immune derangements observed in sarcoidosis, we evaluated serum interferon and immune complex levels in 45 patients with active disease. In none of our patients could circulating interferon be detected, suggesting that an infectious (viral) etiology is very unlikely, and that a great difference exists between sarcoidosis and true autoimmune diseases. On the other hand, circulating immune complexes could be found in 64.4% of our patients. A good correlation could be found with disease stage and duration, but only with ^{67}Ga lung scan among other activity indexes.

Introduction

Sarcoidosis is a chronic granulomatous disorder of unknown cause, characterized in early stages by alveolitis and high levels of activated T cells [1] belonging to different subsets; these cells probably display helper activity [2] in lung and suppressor activity in peripheral blood [3, 4]. Activated cells could support both granuloma formation in the lung (by recruiting circulating monocytes) and delayed immunity impairment in peripheral blood [5]. Many soluble factors could explain T cell activation [6] and attention has been focused on circulating immune complexes (CIC) [7]. On the other hand, the possible role of interferons (IF) as soluble mediators of T cell activity cannot be discarded [8]. γ-IF, a glycoprotein produced by activated T cells, possesses important modulating influences on both cellular and humoral immune mechanisms. It can impair antibody synthesis [8] and lymphocyte proliferation [9], enhance macrophage proliferation, phagocytosis and cytocidal properties [10]. In order to assess these hypotheses, we evaluated serum IF levels and CIC in 45 patients (20 men, 25 women, aged 22–46 years) with histologically confirmed sarcoidosis. Both immune complex and IF levels (evaluated respectively by conglut-

inin binding assay and vesicular stomatitis virus cytopathogenic effect inhibition) were correlated to disease duration and activity.

Materials and Methods

All blood samples were drawn after at least 1 month of corticosteroid therapy interruption. Sera were immediately separated and stored at −20 °C until used.

CIC Assay

CIC were determined with conglutinin binding assay, according to *Manca* et al. [11]. Results were expressed as inhibition coefficient (IC), according to the following calculation:

$$IC = \left(1 - \frac{\text{sample absorbance}}{\text{negative control absorbance}}\right) \times 100.$$

Samples showing an IC >36 were considered as positive.

Circulating IF Assay

50 µl of each serum sample, diluted 1:1 with Eagle's MEM without serum, were placed in the first well of each row of a flat-bottom microtiter plate containing a monolayer of WISH (human amnion) cells. Each sample was then diluted twofold up to 1:32. Another aliquot of each serum sample was acidified with HCl 0.1 *N* (pH 2; 6 h at 4 °C), brought to pH 7.4 with NaOH 0.1 *M*, and processed in the same way, in order to assess acid lability. Each plate also contained a twofold dilution of an international standard of IF (kindly supplied by Prof. *A. Pugliese*, Institute of Microbiology, University of Turin). After 18 h incubation all culture medium was discarded, plates were extensively washed with Eagle's MEM without serum, and 100 µl of a vesicular stomatitis virus suspension in Eagle's MEM, 2% FCS, at a multiplicity of 5×10^5 TCID/ml was added to each well. After 24–48 h incubation under the same conditions, IF levels were calculated as the reciprocal of the dilution of each serum protecting 50% of the cell layer from virus cytopathogenic effect, and expressed as international units (IU). IF levels of 4 IU or less were considered as non-significant.

Patients

Patients (20 men, 25 women, mean age 38.7 ± 16.2) were divided into four groups according to the radiological stage (0 = no evidence of intrathoracic involvement; 1 = hilar adenopathy without parenchymal changes; 2 = hilar adenopathy and diffuse parenchymal changes; 3 = diffuse parenchymal changes with fibrosis) and in three groups according to the disease duration: group A – acute disease (duration 2 months); group B – subacute disease (duration 6–12 months), and group C – chronic disease (duration 24 months). In 37 patients ^{67}Ga lung scan, in 32 patients serum angiotensin-converting enzyme (ACE) level and in 40 patients serum IgG level were evaluated as activity indexes.

Results

Circulating IF

No IF activity could be found in most patients or in healthy controls. A nonsignificant IF level could be detected in 4 patients (4 IU).

CIC and Radiological Stage

CIC could be found in 29 of 45 patients (64.4%) with mean IC values of 47.5 ± 13; CIC-negative patients and healthy controls showed mean values of 28.3 ± 4.6 and 24.2 ± 6.4, respectively. The level of immune complexes was found to be high in 14 patients in stage 2 (63.6%), in 8 patients in stage 1 (61.5%) and in 3 patients in stage 0 (50%). No evidence of CIC appeared in healthy controls. The only 4 patients in stage 3 were all positive ($p < 0.01$) (fig. 1).

CIC and Disease Duration

70% of patients with recent active disease showed evidence of CIC, as compared to 87.5% of patients with chronic active disease and to 47% of patients with chronic stable disease ($\chi^2 = 9.0$; $p < 0.01$) (fig. 2).

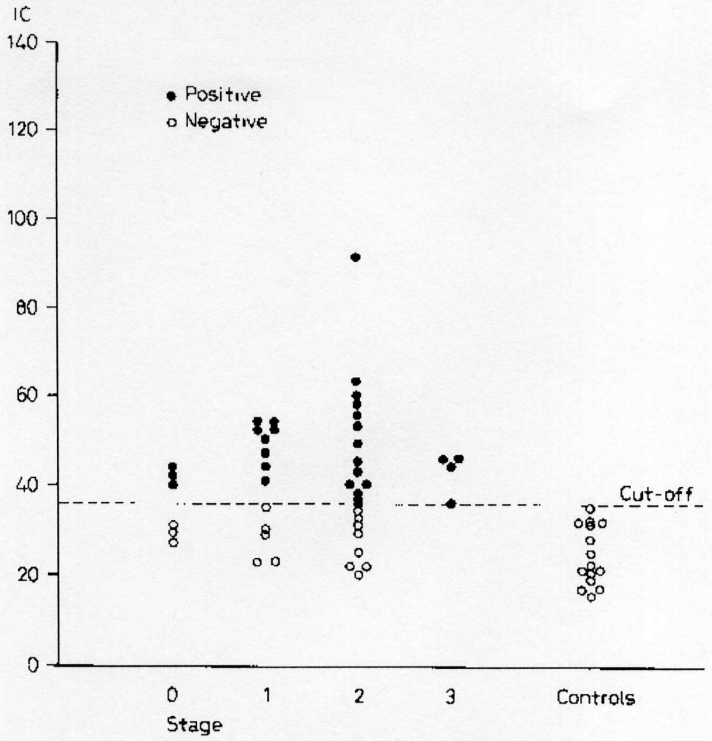

Fig. 1. CIC (conglutinin binding). 16/31 positive (mean IC = 45.2 ± 13); 15/31 negative (mean IC = 27.3 ± 4.6); 11 controls all negative (mean IC = 24.2 ± 6.4).

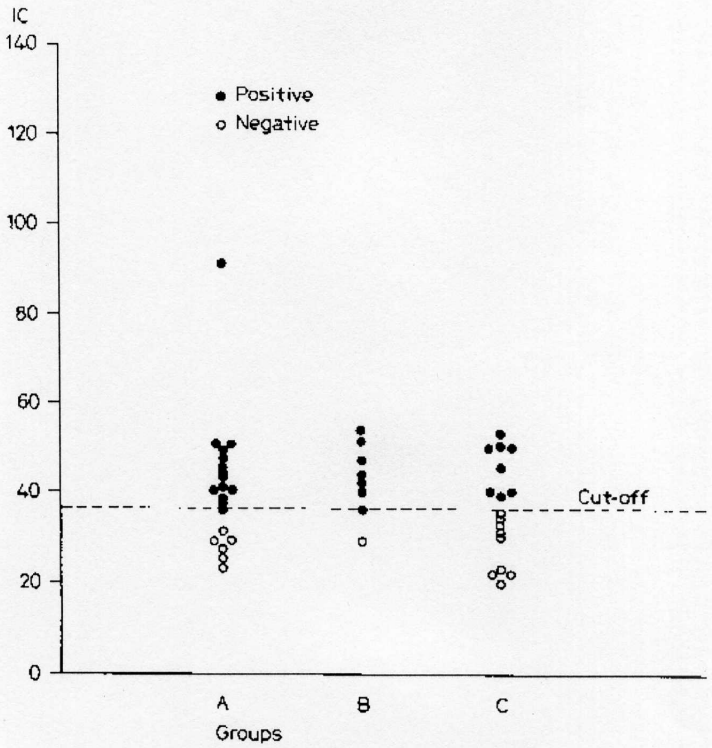

Fig. 2. CIC and disease duration.

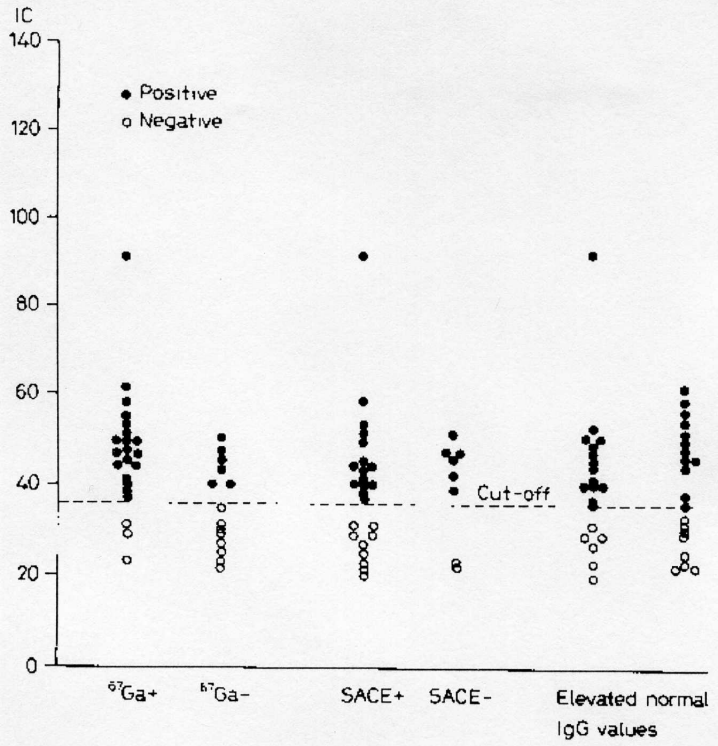

Fig. 3. CIC and disease activity.

CIC and Disease Activity

Disease activity evaluated by ^{67}Ga lung scan (positivity = parenchymal and/or mediastinal uptake ≥ liver uptake) showed a fairly good correlation with CIC detection; indeed, 19 out of 22 Ga-positive patients (86%) showed evidence of CIC whereas in only 6 out of 15 Ga-negative patients (40%) immune complexes could be detected ($\chi^2 = 8.1$; $p < 0.01$) (fig. 3). Serum ACE and IgG levels were also considered as activity indexes. ACE (radiochemical method, Sorin; normal values 125 Units) was determined in 22 patients, and high levels were detected in 24 of them (75%). CIC could be detected in 15 patients showing high ACE levels (62.5%; $p < 0.5$) (fig. 3). Serum IgG level was determined in 40 patients; 20 of them (50%) showed levels above normal. CIC were detected in 14 patients with high serum IgG (70%) and in 12 patients with normal serum IgG (60%) ($p < 0.5$) (fig. 3).

Discussion

Although sarcoidosis possesses some affinities with autoimmune diseases, a striking difference emerging from this work must be emphasized. True autoimmune diseases are often characterized by elevated serum γ-IF levels, probably due to a widespread T-cell activation [12, 13]. Despite a great number of peripheral and lung T cells showing evidence of activation in sarcoidosis have been described by many authors, we could not find any significant level of IF in our patients' serum. This result cannot be due to method sensi-

tivity, as cytopathogenic effect reduction assay is considered to be one of the most sensitive assays [14]. It is also very unlikely that sera storage at −20 °C could damage γ-IF, since serum itself is a good stabilizing agent, and γ-IF seems to be quite heat stable. CIC were detected in 64.4% of patients. Their detection was significantly correlated to disease duration, with a higher frequency of detection in patients with active disease during from at least 6–12 months. Immune complexes could be found also in sera from patients with disease of longer duration, although with a significantly lower frequency than that observed in patients with acute or subacute disease. A significant correlation emerged also between CIC and stage. Indeed, a higher frequency of CIC detection was observed in patients with disease in stage 2, and significantly lower frequencies were found in other stages. While some investigators [15, 16] found higher frequencies of CIC detection in subacute sarcoidosis, others [7, 17] found the highest frequency in chronic disease. Our data are in agreement with the first of them, adding more evidence that different kinds of immune complexes are detected by different assays. This would suggest that a panel of CIC assays should always be employed when studying sarcoidosis. On the other hand, we can confirm that the radiological stage is significantly related to immune complex detection, with higher frequencies of CIC in second stage, as stated by many authors [17–19]. Serum ACE, IgG level, and ^{67}Ga lung scan are now considered to be reliable indexes of disease activity [20, 21]. We found high levels of CIC in 62.5% of patients showing high serum ACE, and in 50% of patients with elevated serum IgG, but no correlation emerged between these activity indexes and CIC detection. On the other hand, ^{67}Ga lung scan positivity appeared to be significantly associated with a high frequency of CIC detection (76% of ^{67}Ga-positive; 40% of ^{67}Ga-negative patients). Our findings support the hypothesis that CIC detection could help the clinician for evaluation of the clinicoradiological stage as well as of disease activity. Indeed, although elevated serum ACE and IgG levels seem to be unrelated to CIC detection, as stated by some authors [1, 17] and confirmed by our work, we can now state that ^{67}Ga lung scan positivity is significantly related to a high frequency of CIC detection in serum. This study lends further support to the belief that CIC detection in sarcoidosis appears to be a useful index of activity and an aid for a better classification.

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